Midge-trapping behaviour and floral biology of *Theriophonum crenatum* Blume. Rumph. (Araceae)

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The species *Theriophonum crenatum* is a rare aroid collected from the open forest of Bilaspur (MP). It exhibited a crepuscular pollination syndrome and monophily towards a midge, *Ceratopogon* sp. The midges get trapped during the night hours, locked the following day, and released during the next day after having been dusted by the pollen released from the collar of male flowers. As many as 380 midges have been collected from a single blossom. The blossom is bisexual and reveals protogyny to avoid self-pollination.

THE pollination of flowers by flies is common. More than 555 species of flowers have been reported to be visited by flies[1]. Similarly, a large number of flowering plants are reported to be visited by mosquitoes[2–4]. However, relatively few species reveal pollination by midges. Knoll[5] has reported pollination of *Arum conocephalloides* by small blood-sucking midges. The plant produces a deceptive odour of the skin of mammals. Kevan and Baker[6] while reviewing flower-visiting insects, do not mention anything about the midges as pollinators. This makes midge pollination an interesting study. The study is important also because the plant is included in the list of rare species by Sivadasan[7]. Among the ariods, while *Sauromatum guttatum* is marked by polyphily[8], *Plesmonium margaritiferum* is reported as pollinated by beetles showing oligophily[9], the species *Theriophonum crenatum* exhibited monophily towards *Ceratopogon* sp. (Ceratopogonidae). On an average 292 midges are collected almost every day in August during the peak of the rainy season. The plant reveals a crepuscular pollination syndrome. The anthesis begins in the evening (5:30 pm) with the release of stench.

The plant produces an average of 9 to 12 flowers per season per plant. It releases a stench of cowdung in the evening which gradually increases up to midnight. Its character of monophily and crepuscular pollination makes it a highly specialized resource-restricted species, which needs a detailed study of floral biology, diel periodicity and mechanism of midge pollination.

The plant is a tuberous herb, with 10 to 15 cm long hastate or sagittate leaves. The experimental plants were brought from degraded forest sites and from grassy fields of Bilaspur (22°12′N, 82°18′E) and grown in a simulated condition in the University plantation site at Rewa (24°32′N, 81°18′E). A colony of 236 plants in 7 m^2 experimental area grew over a period of six years and naturalized in the area. The studies of floral biology and pollination were conducted during the rainy seasons of 1994–95 and 1995–96.

The changes in the blossom were noted at hourly intervals from the day buds first open to the day the blossom shows signs of fading. Blossom was observed for five min during each hour throughout the day and night to determine the nature of its anthesis. The midges were sampled from the sacrificed flowers at hourly intervals to determine their peaks of densities and their locking, trapping and release behaviour by the blossom.

Two kinds of operations were made to study the nature of pollination and seed set. In the first experiment, the blossom was excluded from the possible visits by midges through covering it with plastic bags. At least 12 such blossoms covered by the bags and the development of seeds was followed as the blossom matured. In the second experiment, the appendix was removed and girdle of male flowers was emasculated, leaving behind the female flowers to mature in the absence of column brought forward by the insects or by the release from the same blossom. In both the cases blossoms deteriorate, showing the necessity of cross pollination and presence of appendix which releases insect attractant, i.e. cowdung odour. In the experiment in which only male girdle of blossom was emasculated, successful seed development was observed.

Figure 1 gives three stages of floral kinesis of *T. crenatum*, viz. the trapping of midges (Figure 1 *a*), the locking (Figure 1 *b*) and the release (Figure 1 *c*). The spadix consists of a lip of spathe with crenate purple margins; and chamber, enclosing female and sterile flowers. The enclosed axis of inflorescence within the spathe consists of a terminal appendix, a girdle of male flowers within the neck region, and a bunch of sterile flowers within the chamber. The lip of spathe consists of yellowish green with crenate purple margins. In the neck region, the girdle of male flowers leaves sufficient space laterally for the entry of insects. The blossom develops a collar of purple colour in the neck region which gradually fades after some time. First leaves appear with the onset of rains in July. The leaves show a great variation of shape. In some of the plants the leaves are broad and hastate, in others the lobes are much narrower.

The first flower appears after seven to eight days of vegetative growth. It takes three to four days for a bud to mature into a full blossom. The anthesis is crepuscular and the blossom is active during the night followed by a period of stasis next day. The floral kinesis is resumed in the evening next day. On the first night the flower is in the female stage, on the second night anthers mature and release the pollen showing the male stage of development.

The following is the detailed account of the stages of floral movements which these blossoms reveal.

The anthesis. It starts at 17:30 h in the evening in July as rains stabilize. The opening of blossom is marked by the release of appendix and stench. By 18:00 h the appendix is completely out, leaving sufficient space around it in the neck region for the entry of insects. Although the anthesis is crepuscular, the entry of midges within the blossom is nocturnal. The first midges were collected from the blossoms not earlier than 21:15 h. The entry of midges is synchronized with the release of cowdung odour by the appendix.

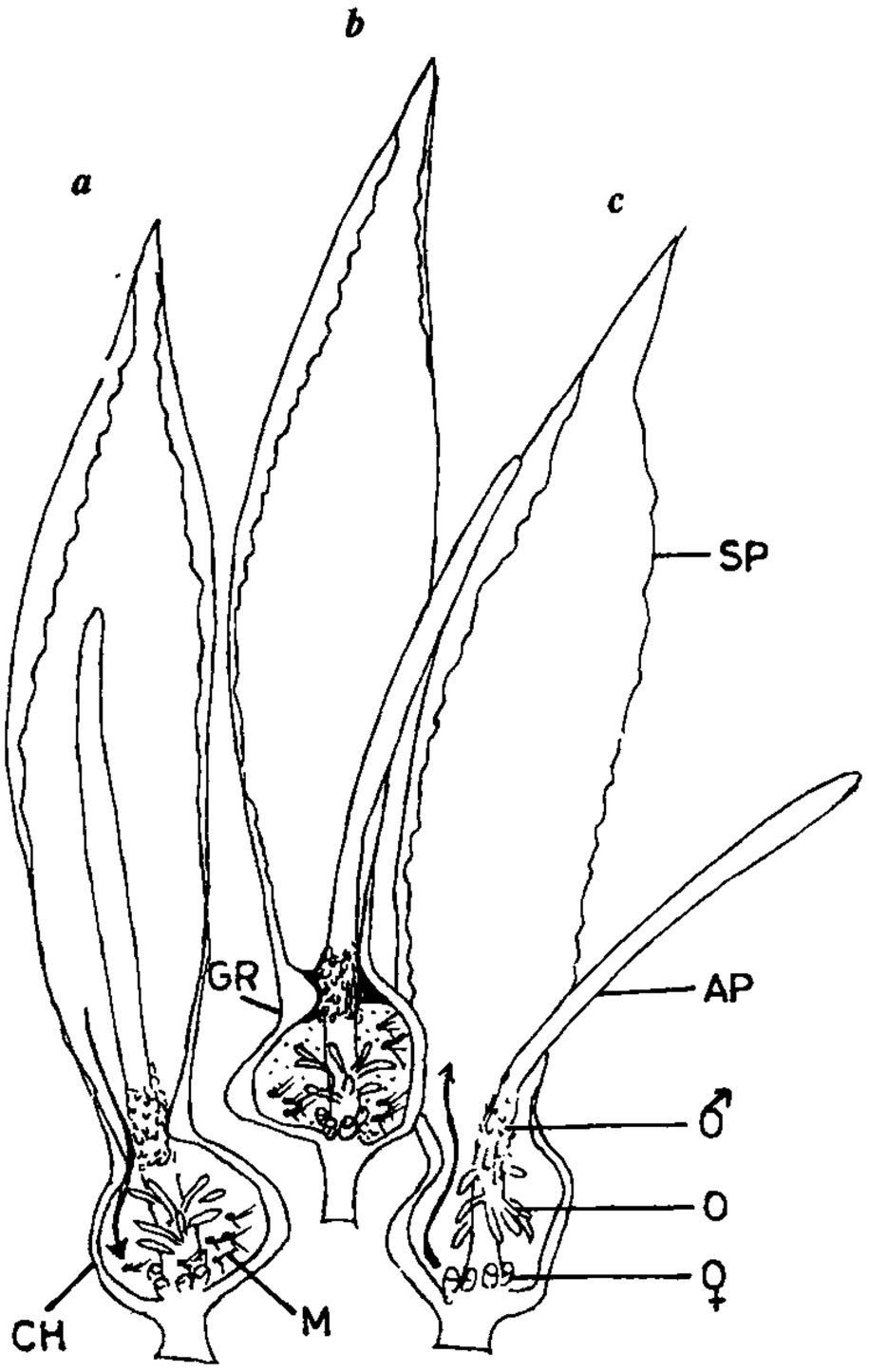

Figure 1. Three stages of flower behaviour, (*a*), trapping; (*b*), locking and (*c*), release of insects. AP = appendix, CH = chamber, GR = grip, SP = spathe, ♂ = male flowers, ♀ = female flowers, O = neutral flowers, M = midges.

Table 1. Diel periodicity and the events of anthokinematics

Time	Temp. (°C)	Events
17:30	27.4	Beginning of anthesis, gradual opening of blossom, stench release.
18:00	27.1	Anthesis complete, blossom fully open, female flowers reach maturity, stigma sticky, anthers undehisced, spathe pale green with a light band of purple colour at the throat.
21:15	26.0	Setting of visits by the insects.
05:00	23.2	Band gets darker, appendix geniculate, tightening of grip by the lobes of spathe against the appendix, locking begins, stigma cuspidate.
06:00	23.8	Locking complete: entry and exit of insects mechanically impossible.
06:10–17:30	24.0–27.5	Stasis: period of suspension of floral kinesis.
18:00	27.0	Resumption of floral kinesis, initiation of pollen rain and pollen dusting of insects.
19:30–21:00	26.5–26.0	Release of grip of appendix by the lobes of spathe, setting of release of insects.
24:00	25.5	Release of pollen-dusted insects complete.

The trapping. (Figure 1 *a*) The pollination syndrome appears to be on the principle of deceit. The blossom offers neither nectar nor pollen to the visiting midges. The release of odour creates the stimulus which attracts the midges towards the floral chamber. The insects fall inside it as there is sufficient space between the neck and the appendix, the latter keeps straight. The entry of insects is nocturnal and takes place after 3 h of anthesis (Table 1).

The blossom reveals protogyny. While staminate flowers are still immature, the pistillate flowers reveal the sticky and shining stigmas, which, however, become cuspidate and dry on the following day. It means that the female flowers are receptive only during the night, on the first day.

The locking. (Figure 1 *b*) The midges fall within the floral chamber and get locked, as there is complete darkness outside the blossom in the night. The midge population reaches its peak in the morning hours. The exit of midges is, however, checked by the development of a deep band of purple colour at the neck region and a downward tilt of the appendix which thereby closes the neck. The development of this band cuts off light from above and checks the exit of midges outside the blossom, who hammer their heads against the pale green translucent walls of the floral chamber. This kind of situation is described as the window pane effect[2,4]. The closure of neck is further achieved by the movement of lobes of spathe which clasp around the appendix, thus securing a perfect trap for the midges during the day time. The insects remain in this captive condition till evening. Virtually the entire activity of blossom ceases during the day time and the stasis prevails.

Pollen rain and release. (Figure 1 *c*) During the evening at 18:00 h the floral kinesis is resumed once again. Anthers mature to release the pollen from the anther slits which are wide open at the top, near the extended connectives. The pollen rains from the staminate girdle and dusts the midges trapped inside. The release of these pollen-dusted insects could be realized by 21:00 h (Figure 2). The insects thus remain locked within the blossom for almost 24 h.

The mechanism of pollination in the species is different from the one described for *Sauromatum guttatum*[10] and *Plesmonium margaritiferum*[9]. The former species revealed the sapromyophily while the latter exhibited cantharophily, the fly pollination and the beetle pollination respectively. While *S. guttatum* is shown to be diurnal in pollination[10], the *T. crenatum* is crepuscular in anthesis

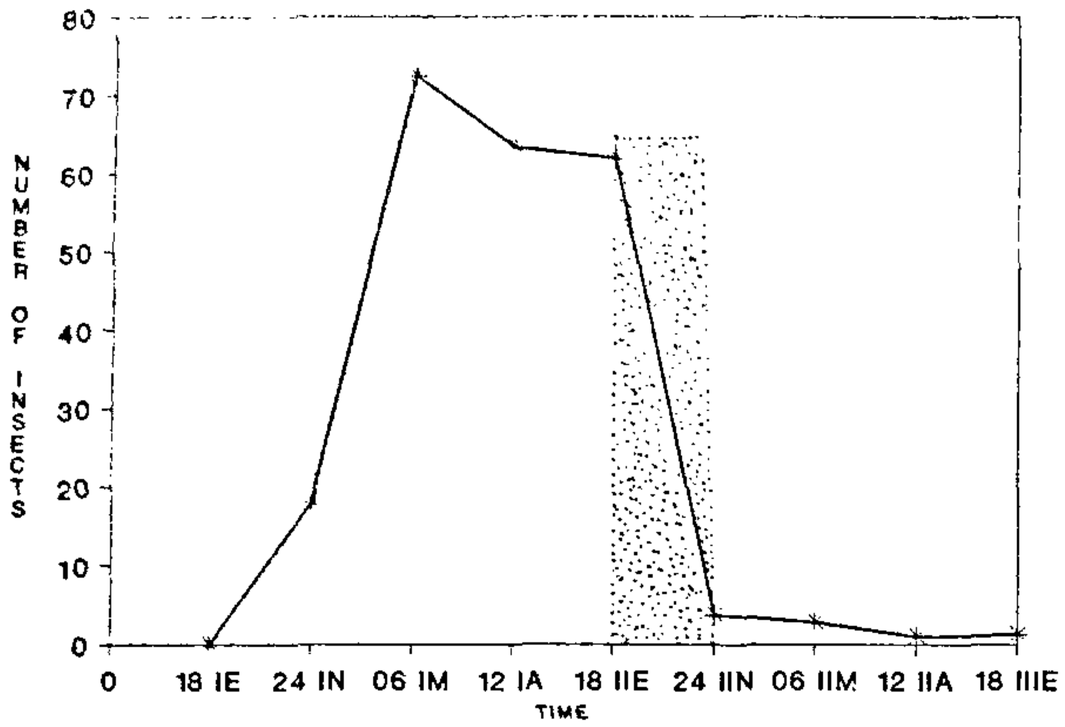

Figure 2. Diel movement of density of insects in the blossom: stippled area reveals the period of pollen rain (E = evening, N = night, M = morning, A = afternoon).

and nocturnal in pollination. In this character it resembles the beetle pollinated *P. margaritiferum*. Since the blossom trapped the blood-sucking female midges, its floral biology is also interesting from the angle of population studies.

1. Kearns, C. A. and Inouye, D. W., *Am. J. Bot.*, 1994, **81**, 1091–1095.
2. Proctor, M. and Yeo, P. F., *The Pollination of Flowers*, Collins, London, 1973.
3. Richards, A. J., *The Pollination of Flowers by Insects*, Academic Press, New York, 1978.
4. Faegri, K. and Van Der Pijl, L., *The Principles of Pollination Ecology*, Pergamon Press, Oxford, 1979.
5. Knoll, F., *Osterr. Bot. Z.*, 1923, **72**, 246–254.
6. Kevan, P. G. and Baker, H. G., *Annu. Rev. Entomol.*, 1983, **28**, 407–453.
7. Sivadasan, M., in *An Assessment of Threatened Plants of India* (eds Jain, S. K. and Rao, R. R.), Botanical Survey of India, Calcutta, 1983, pp. 251–255.
8. Dakwale, S., Ph D thesis, School of Environmental Biology, A.P.S. University, Rewa, 1986.
9. Bhatnagar, S., in *Perspectives in Ecology* (eds Singh, J. S. and Gopal, B.), Jagmindar Book Agency, New Delhi, 1989, pp. 253–270.
10. Dakwale, S. and Bhatnagar, S., *Curr. Sci.*, 1985, **54**, 699–702.

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Photoautotrophic shoot culture: An economical alternative for the production of total alkaloid from *Catharanthus roseus* (L.) G. Don.

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An attempt was made to device an economical alternative for the production of medicinally important indole alkaloids in tissue culture of *Catharanthus roseus* (L.) G. Don. Photoautotrophic shoot cultures were established in liquid medium with cotton fibre as a supporting agent in an indigenously designed culture vessel. Autotrophic cultures, which have the potential of a cost-effective system, produce 10% more total alkaloid as compared to mixotrophic cultures.

CULTURED plant tissues usually grow in a mixotrophic mode which use both CO_2 from air and organic carbon source (mostly sucrose) from the medium. In principle, photoautotrophic shoot culture does not require any sugar in the medium, and uses CO_2 as the sole carbon source. Carbon metabolism is essential to all cells and the nature of carbon source (sugar or CO_2) may affect secondary metabolism and the production of useful compounds[1].

Photoautotrophic shoot cultures of *Catharanthus roseus* (L.) G. Don. have not previously been reported. Interest in photoautotrophy stems from the study of high value indole alkaloids in shoot cultures of periwinkle. The dimeric alkaloids, vincristine and vinblastine could be extracted from mixotrophic shoot cultures, but the yield is very low, possibly because of improper development of chloroplasts due to altered carbon metabolism. Autotrophic shoot cultures contain well-developed chloroplasts. The possibility that would stimulate normal metabolism in leaves and synthesize and accumulate these compounds in higher amounts[2] was apparent.

A two-tier vessel was constructed indigenously with two 250 ml conical flasks according to Husemann and Barz[3] with minor modifications (Figure 1). The upper compartment functions as culture vessel, where the *in vitro* raised shoots[4] were kept on cotton support suspended in 60 ml of sugar-free Murashige and Skoog's (MS)[5] liquid medium supplemented with 6-benzyl aminopurine (0.2 mg/l) and naphtheleneacetic acid (0.1 mg/l). Three multiple shoots, each containing three shoots per clump were used as inoculum.

The lower compartment contained 50 ml of a 2 M $KHCO_3$–K_2CO_3 buffer mixture for enriching CO_2 (2% v/v) in the gaseous atmosphere of the entire culture vessel as suggested by Johnson *et al.*[6]. All openings of the culture vessel were tightly closed with cotton plugs followed by sealing with aluminium foil and finally with cling film to check gas exchange. The culture vessels containing photoautotrophic cultures were incubated in 16 h photoperiod under the light intensity of 42–45 $\mu mol\ m^{-2}\ s^{-1}$ and temperature of $25 \pm 2°C$. The

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